

**Competitive advantage of *Rumex obtusifolius* L. might increase in
intensively managed temperate grasslands under drier climate**

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Abstract

Climate models predict decreasing amounts of precipitation for future summers in Switzerland. Since grasslands cover about one quarter of the area, severe consequences might be expected for Swiss agriculture, ranging from loss of grassland productivity to changes in vegetation composition. Since stressed ecosystems are also more susceptible to invasion, future drier conditions might favour the emergence of weeds. However, the response of temperate grasslands to drought has not been investigated in great detail so far. Using transparent rain shelters, we simulated extreme summer drought conditions in intensively managed temperate grassland in the Swiss lowlands at 400 m (Chamau, located near the city of Lucerne) and studied the drought response of *Rumex obtusifolius*, one of the most troublesome weeds for forage production. We quantified above-ground biomass and assessed the resource use in terms of carbon, nitrogen and water. *R. obtusifolius* increased its above-ground biomass production in response to drought, comprising up to 80% of the total community biomass in 2006. Within the drought plots, highest pre-dawn leaf water potentials, high values for midday leaf water potentials, stomatal conductance and assimilation clearly indicated that *R. obtusifolius* was much less affected by drought than other plant species. In general, no significant differences were found for these variables between drought and control *R. obtusifolius* plants, in contrast to the other plant species. Higher water use efficiency together with a change in N acquisition patterns resulted in a competitive advantage of *R. obtusifolius* over other species, favouring the spread of this weed. Thus, our results suggest a potential increase of weed pressure by *R. obtusifolius* under future climatic conditions, demanding additional management measures to limit its success.

Key words: Climate change, drought, gas exchange, $\delta^{15}\text{N}$, weed, invasion

1 Introduction

Recent climate models predict that until the end of the 21st century the climate in Central Europe will be characterised by further increasing temperatures, changing precipitation patterns and more frequent extreme events such as heat waves and droughts (Schär *et al.*, 2004). Although the projections for precipitation still show much larger uncertainties than those for temperature, most models agree on the general trend of decreasing summer precipitation in Central Europe (Christensen *et al.*, 2007). The very hot and dry summer 2003 is considered a good example of an average future summer by 2100 (Beniston, 2004; Schär *et al.*, 2004). Projections for Switzerland indicate that in 2070, the mean decrease of summer precipitation (June through August) might be around 20% with a maximum decrease of 40% compared to 1990 (Frei *et al.*, 2006), thus leading to severe drought stress, strongly affecting terrestrial ecosystems.

Although water is one of the main resources for plants, most studies investigating the impact of climate change on plant systems have focused on increasing atmospheric CO₂ concentrations (e.g. Leadley *et al.*, 1999; Daepp *et al.*, 2000) or increasing temperatures (e.g. Tungate *et al.*, 2007). In contrast, little is known about the response of temperate ecosystems to the projected changes in precipitation, although the problem was recognised a long time ago (Seifert, 1948). Furthermore, drought effects have rarely been studied in the field, despite their prominent impacts on plant composition, productivity and forage quality. Most of the studies were either model based (e.g. Armstrong and Castle, 1992; Mangan *et al.*, 2004) or small-scale pot experiments performed in a greenhouse (e.g. Patterson, 1988; Karsten and MacAdam, 2001). In addition, the focus was often on semi-arid (e.g.

Whitford *et al.*, 1995) but not on temperate ecosystems, since the problem of drought is more obvious and urgent in those regions that experience water deficits already today (but see Jones *et al.*, 1980a, b). Some long-term studies benefited from naturally occurring droughts and compared the response of the ecosystem in those years to years with average precipitation (e.g. Hopkins, 1978; Silvertown *et al.*, 1994; Buckland *et al.*, 1997; Stampfli and Zeiter, 2004; Hobbs *et al.*, 2007). However, manipulative field experiments simulating severe droughts in temperate grasslands are rare (but see Kahmen *et al.*, 2005; Mikkelsen *et al.*, 2008).

Temperate grasslands are spread over a broad range of management intensities and productivity levels that are closely linked to species composition. More diverse ecosystems (higher number of species or functional types) are thought to stabilise ecosystems functioning (MacArthur, 1955; Pimm, 1984). For example, more diverse grasslands were found to better resist drought (Frank and McNaughton, 1991; Tilman and Downing, 1994; Tilman *et al.*, 2006) and invasion (Hector *et al.*, 2001; Tracy and Sanderson, 2004), although conflicting evidence exists (e.g. Levine and D'Antonio, 1999; Pfisterer and Schmid, 2002; Fargione *et al.*, 2003). Nevertheless, meta-studies generally support this diversity-stability hypothesis (Tilman, 1999; Balvanera *et al.*, 2006). However, the majority of intensively managed temperate grasslands are artificial and species-poor ecosystems. They might thus be less buffered against fluctuating climate and invasion by unsown, i.e. weed species. While studies on the effects of drought on weeds in cropping systems are abundant (for a review see Patterson, 1995), studies on the effects of drought on temperate grassland weeds are lacking, a logical consequence of the missing drought experiments in temperate grasslands.

Successful weeds and invaders are generally associated with certain traits (fast establishment and rapid growth, early reproduction, large number of offspring, Rejmanek and Richardson, 1996). A species combining many of these traits is broad-leaved dock (*Rumex obtusifolius* L.), an erect perennial forb. It is one of the most troublesome weeds in temperate grassland and crop systems in Central Europe. It can grow 40-150 cm tall and persist for several seasons (Cavers and Harper, 1964). *R. obtusifolius* is typical in resource rich meadows and pastures under intermediate moisture conditions (Landolt, 1977; Lauber and Wagner, 1998). *R. obtusifolius* strongly competes for different resources (i.e. nutrients, water, space and light) with the agriculturally more valuable plant species, and it is toxic for most livestock animals (due to its high content of oxalic acid), thereby reducing quality and quantity of rough forage or silage (Hejduk and Doležal, 2004). Consequently, *R. obtusifolius* is not welcomed in grasslands and is typically removed either manually or chemically. However, once present, the species is very difficult to control or even eliminate in the field (Cavers and Harper, 1964). Many efforts have been made to find management options to control *R. obtusifolius* (Niggli *et al.*, 1993; Hopkins and Johnson, 2002; Zaller, 2004b; Gebhardt *et al.*, 2006; Zaller, 2006b), but the best recommendations suggest avoiding its establishment or removing single plants manually early. Despite being such a vigorous agricultural weed, only very little is known on how the performance of *R. obtusifolius* is affected by climate change and drought in particular (e.g. Niggli, 1985) and about the physiological and ecological strategies of mature plants to withstand severe drought (Cavers and Harper, 1964).

Thus, the aim of the present study was to assess the response of *R. obtusifolius* to a severe experimental drought in intensively managed temperate grassland in Switzerland. Using transparent rain shelters, we addressed the following objectives:

(1) To quantify the response of *R. obtusifolius* to a severe spring/summer drought as well as the contribution of *Rumex* to the above-ground biomass production of the whole grassland community, and (2) to assess the ecophysiological controls of the drought response of *R. obtusifolius*, focusing on carbon, nitrogen and water use.

2 Materials and Methods

2.1 Sites

The experiment was conducted in intensively managed temperate grassland at Chamau in the Swiss lowlands at around 400 m a.s.l. (47°12'37''N, 8°24'38''E). Mean annual temperature between 1961 and 1990 at Lucerne (closest meteorological station that had norm values available, about 20 km away) was 8.8 °C, average May to July temperature for the same period was 15.3 °C. Yearly precipitation sum between 1961 and 1990 was 1171 mm, average precipitation sum for May to July was 419 mm (Begert *et al.*, 2003). The soil type is a cambisol (Roth, 2006). The site has been used for grass silage between 1998 and 2001, after which it was turned into a temporary maize crop for one growing season. The meadow was re-established in 2002. The grassland has been reseeded every year with a seed mixture containing *Trifolium repens* L. (white clover), *Lolium perenne* L. (perennial ryegrass), and *Poa pratensis* L. AGG. (smooth meadow-grass). Despite this regular reseeding of only three different species, there was a total of around 20 species present in the experimental plots. During the study, no reseeding of the experimental plots took place. The grassland was intensively managed with six cuts per year and sheep grazing in autumn in some years. Between 2002 and 2005, the meadow was fertilised with slurry after each cut. During the course of the study, experimental plots have not been fertilised. Before cutting, mean leaf area index (LAI) of the meadow was 3.54 at a mean vegetation height of 30.1 cm (data for 2006 from Stohler, 2006).

2.2 **Experimental design**

The drought experiment was established end of June 2005 and pursued over three growing seasons (2005-2007). Five portable rain shelters excluded rainfall in spring/summer, simulating a severe drought. The tunnel-shaped rain shelters were 3×3.5 m of base area and around 2.1 m high at the peak. They consisted of steel frames that were covered with 200 μ m thick transparent plastic foils (Gewächshausfolie UV 5, folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany) during the entire drought treatment period. A core area of 1×2 m below the rain shelters was established to avoid any direct rainfall on the plots. The same setup has been successfully used in an earlier experiment in Central Germany (Kahmen *et al.*, 2005). Five control plots receiving natural rainfall amounts were established next to the sheltered plots. Care was taken that the rainfall removed by the shelters was lead away from the plots. In 2006, two additional rain shelters and control plots were installed to better cover the site's heterogeneity.

To simulate a severe spring/summer drought we relied on the existing climate scenarios for Switzerland as follows. Using regional climate model projections (Frei *et al.*, 2006), we estimated the best start date and duration of an experimental rainfall exclusion for our site. Accumulated precipitation was simulated for the period 1901 to 2000 at grid point Zurich (the model grid point closest to our site) over a period of six to twelve weeks depending on the start date (J. Schmidli, ETH Zurich, pers. comm.). The model results suggested a rainfall exclusion of 8 to 10 weeks beginning in May to achieve a rain exclusion of 200 mm. To represent an extreme future summer drought as projected towards the end of the 21st century, we thus decided to exclude precipitation for about 10 to 12 weeks. During the study period, weather was rather exceptional. While in 2005, very high precipitation occurred in summer (during the exclusion period), 2006 was a record warm year with very low precipitation in

June/July (as seen in the precipitation excluded) but high precipitation in August. The fraction of total precipitation actually excluded varied between 24 and 42% of annual precipitation (Table 1), thus enabling us to achieve our goal to simulate a severe drought.

Soil moisture was monitored continuously at three depths (5, 15 and 30 cm) below two rain shelters (close to the centre) as well as in two control plots using 20 cm long ECH₂O probes (EC-20, Decagon Devices, Inc., Pullman, WA, USA). Measurements were made every 10 seconds and ten minute averages were logged with a CR10X data logger (Campbell Scientific Inc., Logan, UT, USA). Soil moisture was drawn down almost instantaneously after set-up of the shelters, and stayed low until the rain shelters were removed (Fig. 1).

2.3 Above-ground biomass production

Standing above-ground biomass (including dead and alive plant parts) was harvested at the cutting dates of the surrounding farm six times per year (only three times in 2005). Biomass was collected in 20 × 50 cm collection frames ($n=2$ per plot). While the frames were randomly placed on the plots in 2005, they were installed at fixed locations starting spring 2006. After cutting the vegetation at approximately 7 cm above the soil according to the common management practice on the farm, the two samples were pooled (representing 0.2 m²) for further analysis. Biomass was stored in plastic bags at 4 °C for a maximum of one week until further processing. Total community biomass was separated into species and total dead fractions, dried at 60 °C and dry matter was then determined for each species sample separately. Only the species fractions were used for further isotopic and elemental analyses (see below). Due to the low biomass production of *R. obtusifolius* in 2005, two additional leaf disc samples per treatment, originally taken for physiological analyses (see

below) in October 2005, were analysed for C and N isotope ratio and concentration (see below). In September 2007, three to four individuals of *R. obtusifolius* were analysed separately to gain information on root/shoot allocation patterns.

2.4 Plant physiology

In 2006, four abundant species were selected for physiological measurements: *Phleum pratense* L. AGG. and *Lolium multiflorum* LAM. (grasses), *Trifolium repens* L. (legume) and *Rumex obtusifolius* L. (forb). For each species, four to five leaf replicates (youngest fully expanded leaves) from four to five different plots were analysed. Pre-dawn leaf water potential (Ψ_p) was measured in the early morning before sunrise (between 4 and 5 a.m.), while all other physiological measurements were performed between 11 a.m. and 1 p.m. on sunny days. Pre-dawn (Ψ_p) and midday (Ψ_m) leaf water potentials were measured using a Scholander pressure chamber (SKPM, Skye Instruments Ltd, Powys, UK). Photosynthetic gas exchange measurements were performed using an infrared gas analyzer (IRGA) system (CIRAS-1, PP-Systems, Hitchin, UK). Stomatal conductance (g_s) and net photosynthesis per unit leaf area (A_n) were measured in natural but saturating light conditions. Temperature inside the cuvette was set to 25 °C and relative humidity was always close to ambient conditions.

2.5 Isotopic and elemental analyses

Above-ground biomass samples of the most abundant species (*Agrostis stolonifera* L., *Alopecurus pratensis* L., *Dactylis glomerata* L., *Lolium multiflorum* LAM., *Phleum pratense* L. AGG., *Poa pratensis* L. AGG., *Poa trivialis* L. S.L., *Rumex obtusifolius* L. and *Trifolium repens* L.) were ground to a fine powder. Carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) as well as concentrations of these two elements were determined in a combined measurement using a Flash EA 1112

Series elemental analyser (Thermo Italy, former CE Instruments, Rhodano, Italy) coupled to a Finnigan MAT Delta^{plus} XP isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany) via a 6-port valve (Brooks *et al.*, 2003), a ConFlo III (Werner *et al.*, 1999) and an additional Nafion-trap backed by a conventional Mg(ClO₄)₂-trap followed by a 4-port valve (Werner, 2003) between reduction tube and GC column. Post-run off-line calculations (blank, offset and possibly drift corrections) were performed to assign the final δ -values on the V-PDB and AIR-N₂ scales according to Werner and Brand (2001). The long-time precision for the lab's quality control standard tyrosine (~ 2.5 years) was 0.05‰ for $\delta^{13}\text{C}$, and 0.04‰ for $\delta^{15}\text{N}$.

2.6 Statistics

Statistical analyses of all biomass-related variables were performed using R 2.5.0 (R Development Core Team, 2007). Simple one-way analyses of variance (ANOVA) were used to test the effect of the drought treatment on community and *R. obtusifolius* biomass, isotopic signatures, C and N concentrations of *R. obtusifolius*. To compare isotopic signatures as well as C and N concentrations of *R. obtusifolius* with the other plant species, ANOVA models were used. A contrast variable assigning all samples into two categories (*R. obtusifolius* vs. all other species) was introduced to test differences in the response of *R. obtusifolius* vs. all other species. The treatment was fitted first and tested against the plot residuals. Then, the contrast factor (*R. obtusifolius* vs. all other species) and the interaction term treatment×contrast were fitted and tested against the model residuals. A significant effect of the contrast on the tested variables indicates a difference between *R. obtusifolius* and all other species, independent of the treatment (drought or control). A significant interaction of the contrast with the treatment indicates a contrasting drought response of *R. obtusifolius*

compared to the other species. For all physiological parameters (Ψ_p and Ψ_m , A_n and g_s), statistical analyses were performed using ANOVA models in SAS 8.1 (SAS Institute Inc., Cary, NC, USA), testing the treatment effect for each species separately. Tukey honest significant difference tests were used for multiple comparison of means for the different species within a treatment.

3 Results

3.1 *Above-ground biomass production*

Total annual community above-ground biomass production (i.e. dead and alive parts at time of harvest) did not differ between drought and control plots in 2005 (three harvests only, $P=0.53$) nor in 2006 ($P=0.89$). During the 2005 growing season, above-ground biomass regrowth of the intensively managed grassland community steadily decreased from 245 g m^{-2} in June (control plots) to 86 g m^{-2} in September (drought plots). The drought treatment did not significantly reduce community above-ground biomass regrowth, despite the exclusion of almost 500 mm of rain in 2005 (Fig. 2a), resulting in average above-ground regrowth of drought plots ($142 \text{ g m}^{-2} \pm 23 \text{ g m}^{-2}$) being only slightly smaller than that of control plots ($181 \text{ g m}^{-2} \pm 30 \text{ g m}^{-2}$, $P=0.32$). Similar community drought responses were observed in 2006 (Fig. 2b). During the course of the year, average community above-ground regrowth after the harvests were almost identical for drought and controls plots ($146 \text{ g m}^{-2} \pm 15 \text{ g m}^{-2}$ vs. $147 \text{ g m}^{-2} \pm 15 \text{ g m}^{-2}$, respectively, $P=0.98$), ranging between 232 g m^{-2} in June (drought plots) and 71 g m^{-2} in October (control plots).

These unexpected drought responses in 2005 and 2006, particularly the high above-ground biomass production during the drought in 2006 (Fig. 2b), were accompanied by considerable changes in above-ground biomass production of one plant species, *Rumex obtusifolius* (Fig. 2d, Gilgen and Buchmann, 2008). While there

was hardly any *R. obtusifolius* present in 2005, *R. obtusifolius* contributed up to 80% to community above-ground biomass in some plots in 2006.

3.2 Leaf water potentials

In order to better understand plant responses to drought, we measured plant water relations and gas exchange of *R. obtusifolius* plants. Pre-dawn leaf water potentials (Ψ_p) of *R. obtusifolius* varied between -0.25 MPa and -0.06 MPa when the rain shelters were installed in 2006. During the drought treatment, Ψ_p in drought plots dropped to significantly lower levels than in control plots (Fig. 3a), but this drought response disappeared again quite fast during the recovery phase following the removal of the rain shelters. In contrast to pre-dawn leaf water potentials, midday leaf water potentials (Ψ_m) were always considerably lower than Ψ_p , varying between -1.17 MPa and -1.52 MPa (Fig. 3a, b), but no drought effect could be observed for *R. obtusifolius* (Fig. 3b). Similar drought responses in Ψ_p and Ψ_m were measured at the end of the drought treatment in 2006 for selected grass and legume species (*P. pratense*, *L. multiflorum* and *T. repens*), although their Ψ_p and Ψ_m values were typically significantly lower (more negative) than those of *R. obtusifolius* in both control and drought treatments (Table 2).

3.3 Stomatal conductance and carbon assimilation

Stomatal conductance values (g_s) of *R. obtusifolius* varied between 333 mmol H₂O m⁻² s⁻¹ and 937 mmol H₂O m⁻² s⁻¹ but no significant drought effects could be observed for *R. obtusifolius* plants during summer 2006 (Fig. 3c). In contrast, very pronounced drought responses in g_s were measured for the two grasses (*P. pratense* and *L. multiflorum*), but not for *T. repens* at the end of the drought treatment (Table 2).

Net carbon assimilation rate (A_n) of *R. obtusifolius* was quite stable at around $20 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ throughout the growing season in 2006, and no major differences between drought and control treatments were measured between June and September when the rain shelters were up or shortly after they were removed (Fig. 3d). In contrast, A_n rates in grasses (*P. pratense* and *L. multiflorum*) were significantly lower in drought than in control plots, while *T. repens* again showed a very similar response with no pronounced drought behaviour like *R. obtusifolius* at the end of the 2006 drought period (Table 2).

3.4 Isotopic and elemental analyses

To gain more insight into the resource use of *R. obtusifolius*, we analysed the carbon and nitrogen isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively) as well as C and N concentrations (alive plant parts only). During 2005, when *R. obtusifolius* was still scarce in all plots, $\delta^{13}\text{C}$ values averaged -29.6‰ ($\pm 0.3\text{‰}$ SE) under control conditions, which differed marginally ($P=0.09$) from the average value of -30.7‰ ($\pm 0.5\text{‰}$ SE) under drought conditions (Fig. 2e). However, in 2006, when *R. obtusifolius* became more abundant, $\delta^{13}\text{C}$ values in early summer (May and June, before the rain shelters were installed or shortly after) were significantly higher for *R. obtusifolius* plants growing in drought plots ($-27.1\text{‰} \pm 0.1\text{‰}$ in May and $-27.0\text{‰} \pm 0.2\text{‰}$ in June) than in control plots ($-28.1\text{‰} \pm 0.2\text{‰}$ in May and $-28.0\text{‰} \pm 0.1\text{‰}$ in June). Later in the season (i.e. July and August), $\delta^{13}\text{C}$ values of *R. obtusifolius* tissues that had regrown completely during drought conditions decreased, converging with ($-27.7\text{‰} \pm 0.2\text{‰}$ vs. $-27.7\text{‰} \pm 0.4\text{‰}$ in July) or even being lower ($-28.9\text{‰} \pm 0.2\text{‰}$ vs. $-28.4\text{‰} \pm 0.1\text{‰}$ after 10 weeks of drought in August) than the $\delta^{13}\text{C}$ values of tissues regrown under control conditions. After the rain shelters were removed, no differences in $\delta^{13}\text{C}$ between treatments were observed any longer (Fig. 2f).

In contrast, $\delta^{15}\text{N}$ values of *R. obtusifolius* revealed a much clearer picture. While average $\delta^{15}\text{N}$ values were similar for plants in drought and control plots in 2005 ($11.6\text{‰} \pm 1.7\text{‰}$ and $9.2\text{‰} \pm 0.5\text{‰}$, respectively, $P=0.1$; Fig. 2g), drought generally significantly increased the average $\delta^{15}\text{N}$ values for *R. obtusifolius* plants throughout 2006 (annual averages $10.7\text{‰} \pm 0.4\text{‰}$ vs. $7.8\text{‰} \pm 0.5$, respectively; $P<0.001$; Fig. 2h). This response was also found in 2007, when an additional sampling of *R. obtusifolius* took place (September 2007; Fig. 4). Here $\delta^{15}\text{N}$ values of *R. obtusifolius* above-ground biomass regrown under drought conditions ($14.8\text{‰} \pm 2.3\text{‰}$) were not only significantly higher (by about 7‰ in 2007 compared to about 3‰ in 2006) than for those plants regrown under control condition ($7.2\text{‰} \pm 0.4\text{‰}$; $P<0.001$), but the average $\delta^{15}\text{N}$ value in September 2007 ($14.8\text{‰} \pm 2.3\text{‰}$) was also higher than in September 2006 ($10.3\text{‰} \pm 0.5\text{‰}$; $P=0.05$). In contrast, no such change in the ^{15}N signature could be observed for *R. obtusifolius* plants regrown under control conditions during the two seasons ($7.8\text{‰} \pm 0.3\text{‰}$ in 2007 vs. $7.3\text{‰} \pm 1.0\text{‰}$ in 2006, respectively; $P=1.0$). In root tissues, a large difference in $\delta^{15}\text{N}$ of around 6‰ was also found between treatments in 2007, with higher $\delta^{15}\text{N}$ values under drought than under control conditions ($11.5\text{‰} \pm 2.0\text{‰}$ vs. $5.5\text{‰} \pm 0.4\text{‰}$, respectively; $P=0.01$). These overall patterns observed in *R. obtusifolius* plants regrown under drought conditions in 2007 did not just mirror the $\delta^{15}\text{N}$ of bulk soil, which had an average $\delta^{15}\text{N}$ values of $7.6\text{‰} \pm 0.1\text{‰}$ (drought and control plots), quite close to the ^{15}N signature of control plants (Fig. 4).

Carbon and nitrogen concentrations in above-ground biomass of *R. obtusifolius* plants varied between 41% and 45% for C and between 2.5% and 6% for N, but did typically not differ significantly between drought and control treatments (Fig. 2i to l), despite the quite high N concentrations for plants in drought plots in

October 2006 (Fig. 21). Similar patterns were also observed for above-and below-ground tissues of *R. obtusifolius* in 2007, with N concentrations in above-ground biomass of around 4% and in below-ground biomass of around 1.3%, independent of the treatments (Fig. 4). Throughout the experiment, the ratio of carbon to nitrogen in *R. obtusifolius* above-ground biomass (data not shown) was between 7.4 and 17.0, and was unaffected by drought (except for May 2006). Moreover, C and N pools in *R. obtusifolius* biomass were not affected by drought in 2005 ($P=0.6$ for both pools), while in 2006 drought tended to increase both C and N pools ($32.8 \text{ gC m}^{-2} \pm 7.4 \text{ gC m}^{-2}$ vs. $22.9 \text{ gC m}^{-2} \pm 7.4 \text{ gC m}^{-2}$, $P=0.4$ and $2.3 \text{ gN m}^{-2} \pm 0.5 \text{ gN m}^{-2}$ vs. $1.5 \text{ gN m}^{-2} \pm 0.4 \text{ gN m}^{-2}$, $P=0.2$, respectively).

Comparing *R. obtusifolius* to all other plant species present in the plots (Table 2; contrast) and testing for treatment differences (Table 2; treatment) revealed that the treatments (i.e. drought vs. control) explained the observed differences in N concentration and C/N ratio of the total plant community but not in their C concentration or carbon and nitrogen isotopic signatures (except for September 2006). Particularly for the latter three variables, the different response to drought of *R. obtusifolius* compared to all other species was quite pronounced (Table 2; P values of treatment \times contrast for C concentration, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The average $\delta^{13}\text{C}$ values in above-ground biomass of *R. obtusifolius* were generally less negative than those of all other species, independent of the treatment (except for August 2006). The average $\delta^{15}\text{N}$ values of *R. obtusifolius* were always higher than the $\delta^{15}\text{N}$ values of all other species in the community, independent of treatment and harvest. On the other hand, $\delta^{15}\text{N}$ values of *R. obtusifolius* growing in drought plots differed from those growing in control plots (except for October 2006), while no such differences between treatments were apparent for the other plant species in the grassland community.

4 Discussion

Since water is an important resource limiting plant growth, above-ground biomass productivity of grasslands is typically expected to decrease under drier conditions. Indeed, many studies have documented such a productivity decrease under experimental and natural drought (e.g. Tilman and El Haddi, 1992; Fay *et al.*, 2003; Keller and Fuhrer, 2004; Kahmen *et al.*, 2005). However, our results did not follow this expected pattern. Although we found a trend of decreasing community above-ground productivity under drought conditions in 2005, there was no significant reduction in 2006 but instead a slight increase in community above-ground productivity under drought conditions. This trend could be attributed to an increase in productivity of *R. obtusifolius* while productivity of the other species decreased (average non-*Rumex* biomass harvested was $96 \text{ g m}^{-2} \pm 9 \text{ g m}^{-2}$ in drought plots vs. $117 \text{ g m}^{-2} \pm 9 \text{ g m}^{-2}$ in control plots). Our findings are consistent with those of Hobbs *et al.* (2007) in a Californian grassland community that showed an increase in biomass of a single species (*Microseris douglasii*, an annual forb) after drought. Such behaviour is typical of alien invader species, which generally increase community above-ground biomass (Dassonville *et al.*, 2008). However, *R. obtusifolius* is a native, although unwanted, species in managed Swiss grasslands. Therefore, the species had to be removed manually from our experimental plots in October 2006, based on Swiss legislation. This removal of individual *R. obtusifolius* plants was successful, and regeneration of *R. obtusifolius* in 2007 was very sparse (data not shown).

It is well known that non-optimal management of meadows and pastures (e.g. extended periods of time between cuts that allow *R. obtusifolius* to flower and shed seeds or disturbance of the vegetation cover due to trampling by animals) can lead to spread of *R. obtusifolius* in existing grassland communities. Although we observed no apparent drought effect on *R. obtusifolius* during the first year of the study (2005), the

drought treatment considerably reduced total vegetation cover in spring 2006 (reduction to an average vegetation cover of 75% on drought plots compared to 92% on control plots, $P=0.02$, Stohler, 2006), thereby creating gaps in the vegetation similar to those created by mismanagement. *R. obtusifolius* is a gap filler: its seedlings require bare vegetation patches to emerge (Cavers and Harper, 1964) and its initial growth is strongly suppressed by above- and below-ground competition with other plant species (Jeangros and Nösberger, 1990; Zaller, 2004a). It therefore probably benefited from the increased gap space in drought plots in spring 2006, establishing preferentially on previously drought-treated plots, and further expanding during the drought treatment period in 2006. In a regrowth experiment, *R. obtusifolius* grew highest and quickest under drought compared to well-watered or waterlogged conditions, and the number of shoots and leaves was highest under drought (Pino *et al.*, 1995). Following the fluctuating resource theory of Davis *et al.* (2000), the gaps created by drought can be considered as patches of unexploited resources and therefore increased invasibility. Such higher invasibility of grasslands under drought was indeed found by Dukes (2001), while Burke and Grime (1996) showed that invasibility of communities was correlated to the availability of bare ground.

Plant-water relations play an important role for competition within plant communities. Tap roots reaching down to 2.5 m have been reported for *R. obtusifolius* in temperate grasslands (Kutschera *et al.*, 1992), a further advantage over other species. Under drought, these tap roots might allow access to water sources that were not available to shallow-rooted species, especially grasses, as shown by Zaller (2007). This assumption is strongly supported by less negative leaf water potentials (both Ψ_p and Ψ_m), and higher stomatal conductance in *R. obtusifolius* compared to other species, especially grasses, indicating that *R. obtusifolius* was less affected by the

drought treatment than other species. Many studies have shown that multiple factors (e.g. light, temperature, VPD, intercellular CO₂ concentration and water flow from the soil to the plant) influence stomatal conductance (Larcher, 2003; Tuzet *et al.*, 2003), but that different species can respond differently (e.g. Henson *et al.*, 1989; Tardieu and Simonneau, 1998). Thus, better access to water might also explain why drought had no significant effect on photosynthesis rates of *R. obtusifolius*, in contrast to the other species. Similar observations were reported by Funk and Vitousek (2007) with higher carbon assimilation rates of invasive compared to native species. Further evidence of less water stress can be provided by the ¹³C data. Drought increased the $\delta^{13}\text{C}$ values of *R. obtusifolius* above-ground biomass, indicating lower carbon discrimination, lower mesophyll to ambient air CO₂ concentrations and a higher A/g_s ratio (Farquhar *et al.*, 1989), and higher water use efficiency (WUE). Recently, due to a variety of factors affecting WUE, the use of ¹³C signatures as direct indicators for WUE has been discussed in great detail (Seibt *et al.*, 2008). In our case, direct gas exchange measurements of *R. obtusifolius* showed similar A_n rates but lower g_s values under drought conditions, which points to higher WUE under drought.

The measurements reported here thus indicate that drought affected the performance of *R. obtusifolius* less than other species (e.g. the grasses *P. pratense* and *L. multiflorum*), favouring *R. obtusifolius* in its competition with other species. As a result, *R. obtusifolius* increased its cover and its biomass production, outcompeting other plant species (Oswald and Haggard, 1983) or suppressing growth of other species allelopathically (Carral *et al.*, 1988; Zaller, 2006a). Our findings are also consistent with observations from the extremely dry and hot summer 2003 in Switzerland, which showed that the *Rumex* pollen concentration in the air did not change during this very

special period compared to average years, while the concentration of grass pollen decreased (Gehrig, 2006).

Drought altered not only the water relations of *R. obtusifolius* but also its nitrogen dynamics. N concentrations of *R. obtusifolius* were similar to those of the other species, except in drought plots at the beginning of the growing season. At that time, drought significantly increased N concentrations in *R. obtusifolius* compared to other species, which might have stimulated photosynthesis, thereby initiating the positive drought response of *R. obtusifolius* biomass production observed in May and June 2006. Although this drought effect on N concentrations disappeared quite fast later in the season, the high productivity rates of *R. obtusifolius* resulted in large amounts of N taken up in drought plots, thereby depleting the soil N pool for the other species. Moreover, the $\delta^{15}\text{N}$ values also reflected the contrasted drought response patterns of *R. obtusifolius* compared to other species. The $\delta^{15}\text{N}$ values of *R. obtusifolius* were generally higher than those of other species, and were significantly increased under drought. In contrast, drought had no significant effect on the ^{15}N signature in the other species. The lack of mycorrhizal associations in *R. obtusifolius* (Cavers and Harper, 1964) could explain the difference between this species and the other species of the plant community. Any influence of fertilisation on the $\delta^{15}\text{N}$ values can be excluded because our experimental plots were not fertilised during the experiment, and were under similar management during the years prior to the experiment. Furthermore, drought had no significant effect on the $\delta^{15}\text{N}$ of the soil in autumn 2007, after three years of drought (7.56‰ in both treatments). We thus assume there was no confounding of the plant's signature due to changing background ^{15}N signature of the bulk soil, changed allocation to different tissues, or changes in legume presence with related differences in depleted N from symbiotic fixation.

Consequently, the difference in $\delta^{15}\text{N}$ in *R. obtusifolius* above-ground biomass in drought and control plots reflects a change in N source as a response mechanism to drought. Recently, the difference between $\delta^{15}\text{N}$ values of plant leaves and soil (i.e. $\Delta\delta^{15}\text{N}$) was found to be positively related to the ratio of NO_3^- to NH_4^+ uptake of different species (Kahmen *et al.*, 2008). Thus, the (high) $\delta^{15}\text{N}$ values of *R. obtusifolius* indicate a preference for nitrate over ammonium compared to the other species, particularly in drought plots. In fact, it was suggested earlier that *R. obtusifolius* may be able to take up nitrate more efficiently than other species (Melzer *et al.*, 1984), which is consistent with our ^{15}N data. *R. obtusifolius* was likely able to reach deeper water sources and thereby also a different, more nitrate rich, N source.

Conclusions

In brief, interactions between weed and forage species will be affected by any environmental factor that influences growth and nutrient relations, particularly under future climatic conditions. However, such changes are difficult to predict because they might differ from site to site. Our results support the idea that the competition between temperate grassland species and *R. obtusifolius*, a well-known weed in Swiss managed grasslands, might increase under a future drier climate. A competitive advantage of *R. obtusifolius* is expected to impact forage composition and quality of intensively managed grasslands, depending on site-specific conditions. Any major proportion of bare ground will favour the spread of *R. obtusifolius*, whose plant functional traits, as discussed above, render its management rather difficult. Thus, additional adaptive management measures will be needed in the future to avoid an increased spread of *R. obtusifolius* in temperate grasslands.

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Table 1: Timing of the drought treatment and amount of precipitation excluded at Chamau during the three years of treatment.

	2005	2006	2007
Duration of drought treatment	24.6.–19.9.	31.5.–17.8.	2.5.–10.7.
Days of treatment	87	78	69
Annual precipitation [mm] ^a	1170 ^b	1136	1232
Precipitation excluded [mm] ^a	491 ^b	271	403
Fraction of annual precipitation excluded [%]	42	24	33

^a Precipitation data (unpublished) provided by Matthias J. Zeeman, ETH Zurich.

^b Precipitation data from Chamau is only available from 15.7.2005 onwards. The data was therefore completed using data from the MeteoSchweiz precipitation station Zwillikon (around 10 km away from Chamau) for the period 24.6.–14.7.2005. The two stations showed very good measurement agreement ($R^2=0.78$).

Table 2: Effect of drought on pre-dawn leaf water potential (Ψ_p), leaf water potential at midday (Ψ_m), stomatal conductance at midday (g_s), and CO_2 assimilation rate at midday (A_n) of four selected species (at the end of the drought treatment on 15 August 2006).

Chamae 15 August 2006	Ψ_p [MPa] ^a			Ψ_m [MPa] ^a			g_s [mmol H ₂ O m ⁻² s ⁻¹] ^a			A_n [$\mu\text{mol CO}_2$ m ⁻² s ⁻¹] ^a		
	Control	Drought		Control	Drought		Control	Drought		Control	Drought	
<i>Phleum pratense</i>	-0.22 ± 0.01^a	-0.78 ± 0.03^a	***	-2.17 ± 0.14 ^a	-2.42 ± 0.03 ^a	ns	325 ± 31^a	224 ± 17^a	*	17.2 ± 1.3^a	11.9 ± 1.3^a	*
<i>Lolium multiflorum</i>	-0.21 ± 0.03^a	-0.70 ± 0.05^a	***	-1.94 ± 0.08 ^a	-2.20 ± 0.10 ^{ab}	ns	373 ± 34^{ab}	216 ± 38^a	*	18.8 ± 0.9^a	12.6 ± 0.6^a	***
<i>Trifolium repens</i>	-0.13 ± 0.02^b	-0.26 ± 0.01^b	***	-1.88 ± 0.14 ^{ab}	-1.96 ± 0.03 ^b	ns	799 ± 195 ^b	402 ± 71 ^{ab}	ns	21.5 ± 2.3 ^a	18.5 ± 1.4 ^b	ns
<i>Rumex obtusifolius</i>	-0.07 ± 0.01^b	-0.25 ± 0.01^b	***	-1.44 ± 0.06 ^b	-1.52 ± 0.08 ^c	ns	797 ± 120 ^b	515 ± 54 ^b	ns	19.8 ± 1.1 ^a	18.8 ± 1.4 ^b	ns

^a Means and standard errors are given ($n=4-5$). Significant differences between treatments are given in bold. Species sharing the same letter are not significantly different within the treatment (TukeyHSD). * $0.05 \geq P > 0.01$, *** $P \leq 0.001$.

Table 3: Results of the ANOVA models fitted for stable isotope ratios and concentrations of carbon and nitrogen, respectively, as well as C/N ratios in above-ground biomass (alive plant parts only) in 2006.

Chamau 2006		$\delta^{13}\text{C}^a$	$\delta^{15}\text{N}^a$	Carbon concentration ^a	Nitrogen concentration ^a	C/N ^a
1. harvest 4 May	Treatment	$F_{1,8}=0.47$	$F_{1,8}=0.00$	$F_{1,8}=1.77$	$F_{1,8}=10.5$ *	$F_{1,8}=8.81$ *
	Plot	$F_{8,30}=1.56$	$F_{8,30}=0.68$	$F_{8,30}=0.75$	$F_{8,30}=0.37$	$F_{8,30}=0.56$
	Constrast ^b	$F_{1,30}=48.0$ ***	$F_{1,30}=5.67$ *	$F_{1,30}=0.15$	$F_{1,30}=2.26$	<i>$F_{1,30}=3.08$</i>
	Treatment×Contrast	$F_{1,30}=4.61$ *	$F_{1,30}=0.77$	$F_{1,30}=0.52$	$F_{1,30}=0.64$	$F_{1,30}=0.21$
2. harvest 9 June	Treatment	$F_{1,10}=1.30$	$F_{1,10}=0.00$	$F_{1,10}=0.41$	$F_{1,10}=5.30$ *	<i>$F_{1,10}=4.16$</i>
	Plot	$F_{10,48}=3.41$ **	$F_{10,48}=1.12$	$F_{10,48}=0.71$	$F_{10,48}=0.74$	$F_{10,48}=1.51$
	Constrast ^b	$F_{1,48}=27.4$ ***	$F_{1,48}=18.4$ ***	$F_{1,48}=0.82$	$F_{1,48}=5.43$ *	$F_{1,48}=7.37$ **
	Treatment×Contrast	$F_{1,48}=1.89$	$F_{1,48}=1.22$	$F_{1,48}=4.63$ *	$F_{1,48}=0.15$	$F_{1,48}=0.35$
3. harvest 10 July	Treatment	$F_{1,12}=2.19$	$F_{1,12}=0.18$	$F_{1,12}=0.00$	$F_{1,12}=0.05$	$F_{1,12}=0.00$
	Plot	$F_{12,59}=1.42$	$F_{12,59}=1.94$ *	$F_{12,59}=1.52$	$F_{12,59}=2.11$ *	$F_{12,59}=2.45$ *
	Constrast ^b	<i>$F_{1,59}=3.84$</i>	$F_{1,59}=32.7$ ***	$F_{1,59}=18.9$ ***	$F_{1,59}=0.27$	$F_{1,59}=0.86$
	Treatment×Contrast	$F_{1,59}=0.79$	$F_{1,59}=0.56$	<i>$F_{1,59}=3.54$</i>	$F_{1,59}=0.20$	$F_{1,59}=0.00$
4. harvest 8 August	Treatment	$F_{1,12}=0.25$	$F_{1,12}=1.77$	$F_{1,12}=0.46$	$F_{1,12}=13.7$ **	$F_{1,12}=8.79$ *
	Plot	$F_{12,58}=1.08$	$F_{12,58}=1.37$	$F_{12,58}=2.19$ *	$F_{12,58}=0.62$	$F_{12,58}=0.90$
	Constrast ^b	$F_{1,58}=4.16$ *	$F_{1,58}=36.0$ ***	$F_{1,58}=8.22$ **	$F_{1,58}=1.35$	$F_{1,58}=0.26$
	Treatment×Contrast	$F_{1,58}=1.04$	$F_{1,58}=6.65$ *	$F_{1,58}=0.05$	$F_{1,58}=1.49$	$F_{1,58}=1.43$
5. harvest 5 September	Treatment	$F_{1,12}=6.70$ *	$F_{1,12}=0.02$	$F_{1,12}=2.68$	$F_{1,12}=26.2$ ***	$F_{1,12}=26.0$ ***
	Plot	$F_{12,58}=1.24$	$F_{12,58}=1.16$	$F_{12,58}=0.30$	$F_{12,58}=1.40$	$F_{12,58}=1.11$
	Constrast ^b	$F_{1,58}=9.40$ **	$F_{1,58}=42.8$ ***	$F_{1,58}=19.0$ ***	$F_{1,58}=17.4$ ***	$F_{1,58}=15.1$ ***
	Treatment×Contrast	$F_{1,58}=1.85$	$F_{1,58}=6.76$ *	$F_{1,58}=0.44$	$F_{1,58}=0.73$	$F_{1,58}=1.61$
6. harvest 19 October	Treatment	<i>$F_{1,12}=3.32$</i>	$F_{1,12}=0.07$	$F_{1,12}=0.26$	<i>$F_{1,12}=4.55$</i>	$F_{1,12}=7.39$ *
	Plot	$F_{12,55}=0.58$	$F_{12,55}=0.76$	$F_{12,54}=2.40$ *	<i>$F_{12,55}=1.87$</i>	$F_{12,54}=1.33$
	Constrast ^b	$F_{1,55}=0.01$	$F_{1,55}=4.43$ *	$F_{1,54}=0.21$	$F_{1,55}=29.5$ ***	$F_{1,54}=16.5$ ***
	Treatment×Contrast	$F_{1,55}=6.30$ *	$F_{1,55}=0.25$	$F_{1,54}=5.96$ *	$F_{1,55}=11.6$ **	$F_{1,54}=2.69$

^a $F_{\text{n df, d df}}$ indicates the F value with n df and d df degrees of freedom for the numerator

and the denominator, respectively. Significant values are given in bold, marginally

significant values ($0.1 \geq P > 0.05$) are in italics. * $0.05 \geq P > 0.01$, ** $0.01 \geq P > 0.001$,

*** $P \leq 0.001$.

^b The factor contrast assigns the data into two categories, *Rumex obtusifolius* vs. all other species.

5 Figure legends

Figure 1: (a) Daily precipitation and (b) volumetric soil water content [%] at 30 cm depth at Chamau in 2006. Precipitation data provided by Matthias J. Zeeman (ETH Zurich). Measurements from two plots were averaged for both soil moisture curves. The grey area indicates the period of the drought treatment.

Figure 2: Effect of drought on (a, b) total community above-ground biomass (including dead and alive plant parts), (c, d) *Rumex obtusifolius* above-ground biomass (alive parts only) as well as (e, f) $\delta^{13}\text{C}$, (g, h) $\delta^{15}\text{N}$, (i, j) C concentration and (k, l) N concentration in above-ground biomass of *R. obtusifolius* in 2005 and 2006. Grey areas indicate the periods of the drought treatment. Means and standard errors ($n=1-8$) are given. $\cdot 0.1 \geq P > 0.05$, $* 0.05 \geq P > 0.01$, $** 0.01 \geq P > 0.001$, $*** P \leq 0.001$. *R. obtusifolius* had to be removed manually from the managed grassland plots in October 2006 (see text for details).

Figure 3: Effect of drought on (a) pre-dawn leaf water potential (Ψ_p), (b) leaf water potential at midday (Ψ_m), (c) stomatal conductance at midday (g_s), and (d) CO_2 assimilation rate at midday (A_n) of *Rumex obtusifolius* during the growing season 2006. The grey area indicates the period of the drought treatment. Means and standard errors ($n=4-5$) are given. $** 0.01 \geq P > 0.001$, $*** P \leq 0.001$.

Figure 4: Nitrogen isotope ratios ($\delta^{15}\text{N}$) and N concentrations of *Rumex obtusifolius* above- and below-ground biomass (alive plant parts only) in September 2006 and 2007 (5th harvest in both years). The $\delta^{15}\text{N}$ value of bulk soil is given as for 2007 (dashed line). Means and standard errors ($n=3-6$) are shown. Bars sharing a letter are not significantly different from each other (TukeyHSD, confidence level=95%). All means were compared to each other.

Figure 1

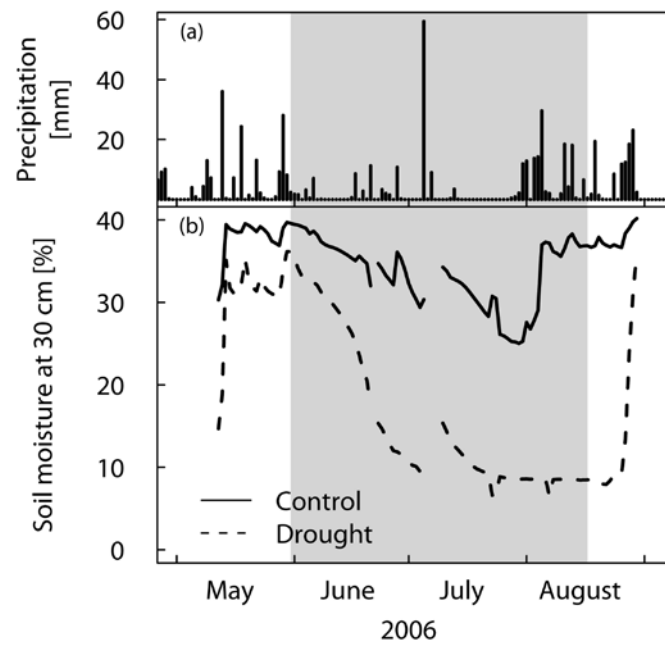


Figure 2

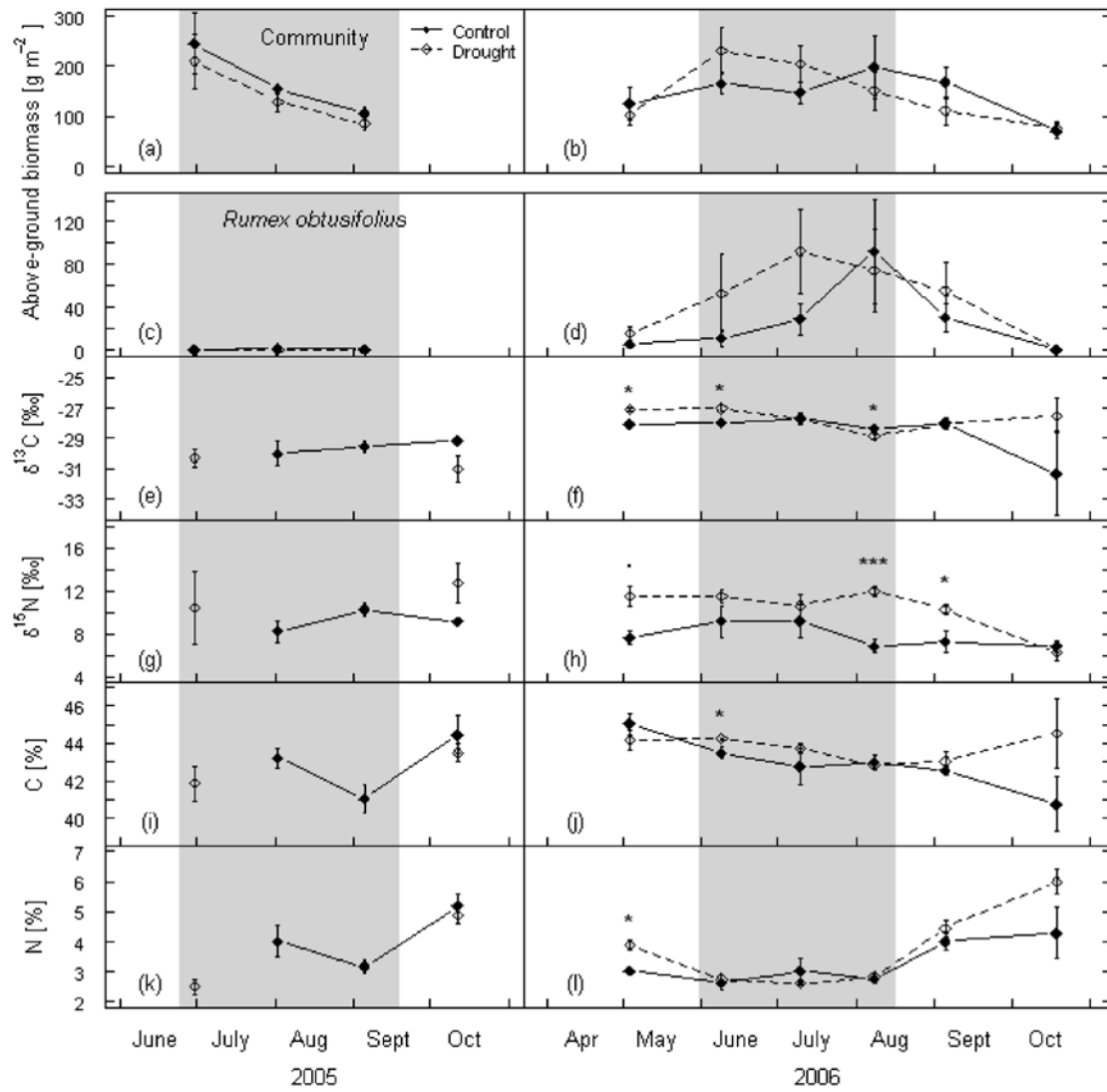


Figure 3

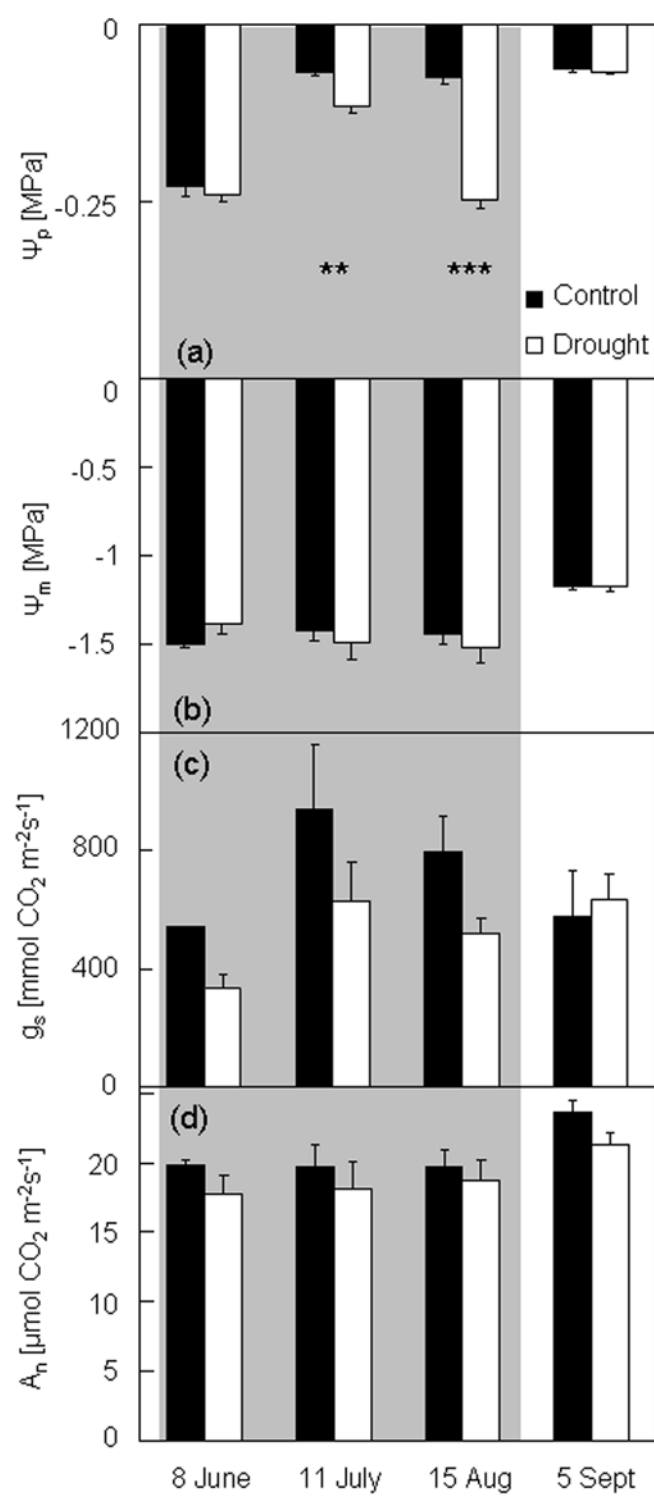


Figure 4

